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Observation of an Fe(III)-OOH Intermediate By XAS: Synthetic Models The Iron Metalloenzyme Superoxide Reductase.

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Beamline: X18B

Introduction: Superoxide reductases (SORs) are non-heme cysteinylated iron metalloenzymes that catalyze the reduction of superoxide into peroxide. This is presumed to occur through a one-electron reduction from Fe^{II} to a bound hydroperoxyl radical, forming an $\text{Fe}^{\text{III}}\text{-OOH}$ intermediate. Protonation of the hydroperoxo ligand followed by displacement of H_2O_2 by glutamate forms the resting ferric form of the enzyme. We have prepared an Fe^{II} synthetic model complex that is oxidized in the presence of HO_2 , affording a stable Fe^{III} complex and H_2O_2 . An intermediate in this reaction can be trapped at low-temperature. Presented is preliminary X-ray absorption data of the intermediate.

Materials and Methods. The iron complex $\text{Fe}^{\text{II}}\text{SN}_4$ was dissolved in MeOH, cooled to -90°C and potassium superoxide was injected in THF as its (18-c-6)[K] salt, affording $\text{Fe}^{\text{III}}\text{SN}_4\text{OOH}$. The resulting solution was then injected into an aluminum sample holder in between two pieces of Kapton tape and quickly frozen. Standards of $\text{Fe}^{\text{II}}\text{SN}_4$ were prepared in a similar manner, except potassium superoxide was neglected. X-ray absorption spectra were obtained using a Si111 monochromator, a Canberra 13-element solid state fluorescence detector and a helium displacer cryostat to maintain the sample at ca. 77 K. Data was measured in 5 eV increments in the pre-edge region (6960 – 7000 eV), 0.5 eV increments in the edge region (7000 – 7171 eV), and 3 eV increments in the EXAFS region (7171 – 7910 eV).

Results. Upon treatment of $\text{Fe}^{\text{II}}\text{SN}_4$ with HO_2 , the Fe K-edge shows a +2.8 eV shift, indicative of an oxidation state change from Fe^{II} to Fe^{III} . The EXAFS region of $\text{Fe}^{\text{II}}\text{SN}_4$ solved well for a five coordinate iron complex with four nitrogen and one sulfur scatterer, with bond-lengths consistent with those previously determined. The EXAFS region of the intermediate solved well for a six coordinate iron complex with four nitrogen, one sulfur, and one short oxygen scatter. Furthermore, a second outer-sphere oxygen scatterer could be located at a distance consistent with an end-bound $\text{Fe}^{\text{III}}\text{-OOH}$ complex. Warming of $\text{Fe}^{\text{III}}\text{SN}_4\text{OOH}$ results in the loss of the outer-sphere oxygen and lengthening of the inner sphere oxygen scatterer.

Conclusions. XAS results are consistent with the intermediate in the oxidation of $\text{Fe}^{\text{II}}\text{SN}_4$ being an $\text{Fe}^{\text{III}}\text{-OOH}$ species. Additionally, electronic absorption, EPR, and NMR studies are all consistent with this being the identity of the intermediate.

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